REMARKS

FORMAL MATTERS

Claims 1-5, 10, 14-20, 22, 27-28, 30-35, and 40 are pending after entry of the amendments set forth herein.

Claims 6-9, 11-13, 21, 23-26, 29, 36-39, 41 are canceled.

Claims 1-5, 10, 14, 15, 27-28, and 32-35 are amended. The amendments to the claims were made solely in the interest of expediting prosecution, and are not to be construed as acquiescence to any objection or rejection of any claim. Support for the amendments can be found throughout the specification, and at, for example, page 19, line 4; and page 18, lines 1-4.

The title is amended as requested by the Examiner.

No new matter is added.

Applicants respectfully request reconsideration of the application in view of the remarks made herein.

INFORMATION DISCLOSURE STATEMENT

The Applicants note that an Information Disclosure Statement and a SB/08A form are being submitted herewith. The Applicants respectfully request that the Examiner initial and return this SB/08A form, thereby indicating that the references cited in the IDS have been reviewed and made of record.

SPECIFICATION

The Examiner states that "[t]he title of the invention will have to be changed to more closely reflect the fact that the claims are limited to a transgenic mouse or rat."

The title has been amended to reflect that the claims encompass transgenic mouse.

REJECTION UNDER §112, ¶1 – ENABLEMENT

Claims 1-5, 8, 10, 13-20, 22, 27, 28, 30-35 and 40 were rejected under §112, ¶1 on the grounds that the claims fail to comply with the enablement requirement. This rejection is respectfully traversed as applied and as it may be applied to the presently pending claims.

The Examiner asserts that the specification and the art at the time of filing do not teach how to make knock in rats or rat ES cells. The Examiner also asserts that the claims encompass using a

heterozygous transgenic knockin mouse and the specification does not teach how to use a heterozygous mouse expressing both human and mouse C5aR.

As amended, the claims recite a transgenic mouse homozygous for the polynucleotide encoding human or humanized C5aR.

As to the §112, ¶1 rejection as it relates to the need for the mouse to be a homozygous knock-out of the endogenous C5aR, Applicants respectfully traverse. It is not necessary that the transgenic mouse be either a heterozygous or homozygous knock-out for the endogenous mouse C5aR gene. The ordinarily skilled artisan would recognize that when such a transgenic mouse was used, then one simply runs an appropriate control. For example, one could simply administer a candidate agent to a control wildtype mouse, and the results compared to those obtained following administration of the same candidate agent to transgenic mouse. Such controls are described in the specification at, for example, Example 6, beginning at specification page 59.

Claim 15

On page 5 of the Office Action the Examiner asserts that claim 15 is not adequately enabled. The examiner asserts that "The specification fails to adequately teach those of skill to make the knock-in mouse described in Example 1".

The reasons for this objection appear to be as follows:

(i) The structure of the targeting construct is not readily apparent from Figure 1 because the region of the "mouse-human fusion" is unclear and does not teach what area of the mouse C5aR has been replaced with human sequences or what promoter is driving the human C5aR sequence

(ii) The targeting vector comprises a cre-lox system which is essential to the invention but not defined in the claims.

Response to (i)

The specification provides clear guidance for generating the targeting construct. For example, at page 52, lines 11 to 32, the specification teaches as follows:

"The mouse genomic region as shown in SEQ ID NO:1 (the target locus) is characterized as follows:

exon 1: nucleotides 757-784 (5' untranslated region)
exon 2: nucleotides 1048-1152 (5' untranslated region plus start codon)
exon 3: nucleotides 10726-11778 (all coding sequence except ATG).

Figure 3 shows a restriction map of the 22kb sequence shown in SEQ ID NO:1. The relevant restriction sites are as follows:

Enzyme	#Cuts	Positions:			
EcoRI	2	18462	19176		
EcoRV	4	199	5337	9151	18964
NdeI	2	7698	18758		
XbaI	4	1351	15968	16852	20902

The targeting vector used to generate the knock-in mice includes regions homologous to approximately 3kb genomic DNA either side of exon 3 (i.e., from about nucleotides 7377-15045 as shown in SEQ ID NO:1). *In particular, the targeting vector comprised the region from about nucleotides 7377-15045 of SEQ ID NO:1 except that nucleotides 10726-11778 were replaced by nucleotides 28 to 1077 of SEQ ID NO:2*." [emphasis added]

Accordingly, this passage of the specification gives a precise description of the mouse-human fusion region shown in Fig. 1.

In view of the above, a skilled person would understand that the control sequences driving expression of the humanized C5aR in the transgenic mouse are the native mouse sequences.

Response to (ii)

Contrary to the Examiner's assertion, the cre-lox system is not essential to the invention. Indeed, the specification does not limit the generation of transgenic mice using the cre-lox system.

First, the specification explains that many selectable markers may be used in the generation of transgenic mice and provides the PGK-neo gene an example of a selectable marker (see page 7, lines 12-13). Hygromycin-B phosphotransferase (HPH) and the Xanthine-guanine phosphoribosyltransferase gene (XGPRT, gpt) are examples of other selectable markers that were both well know to those skilled in the art in 2003.

It was known at the time of filing the present application that if the PGK-neo selectable marker is used, it is preferable to remove it after the targeting construct has been inserted into the genome. This is because the PGK-neo gene has its own promoter which may alter expression of neighboring genes (see the specification at page 51, lines 22-35). Accordingly, because the PGK-neo gene was used as a selectable marker in the exemplified targeting construct, the lox-cre system was used so that the selectable marker could be removed following integration.

Furthermore, a skilled person knows that there are alternatives to the cre-lox system which may be used at the priority date of the present application to remove the PGK-neo gene from the mouse genome. For example, Takeuchi *et al.*, Biochem. and Biophys. Res. Comm. 293: 953-957 (2002) (copy enclosed) describes an Flp recombinase system that was used in a targeting vector in order to remove a selectable marker following integration into a mouse genome. The use of the flp-frt system in mice was first reported in 1996 by Dymecki (Proc Natl Acad Sci U S A. 93(12): 6191–6196 (1996); copy enclosed).

Claim 28

On pages 6 to 8 the Examiner asserts that claim 28 is not adequately enabled. The examiner asserts that the specification fails to enable those of skill to determine how to use the mouse of claim 1 to screen drugs.

The reasons for this objection appear to be as follows:

(i) The specification does not clearly set forth that knock-in mice and wild-type mice were both given K/BxN sera; and

(ii) It was predetermined that the anti-human C5aR antibody targeted hC5aR and not mouse C5aR, so the controls required to identify compounds that specifically target hC5aR using the mice claimed are not described by the Applicants.

Response to (i)

The Applicants respectfully refer the Examiner to the following passages in the specification which describe injection of homozygous C5aR ***and*** wild-type control mice with K/BxN sera:

Page 60, lines 11-15 which states:

> "Sera from arthritic K/BxN mice at 60 d of age were pooled and injected intraperitoneally (i.p.) (150 ul total volume) into 6 – 9 week **old homozygous (H5Rf/H5Rf) and wild-type (+/+) mice** on each of days 0 and +2." [emphasis added]

Page 61, lines 16-18 which states:

> "To induce inflammatory disease **the homozygous human C5aR knock-in mice (on C57BL/6 background) and wild-type/control (C57BL/6) mice** were injected with K/BxN serum." [emphasis added]

Page 62, lines 5-8 which states:

> "Figure 6 shows the increase in ankle thickness and clinical score for **wild-type and homozygous mice injected with the K/BxN serum**. The progression of disease in the homozygous hC5aR knock-in mice was the same as in the control mice with inflammation apparent starting on day 3 after serum injection." [emphasis added]

Figure legend for Figure 6 on page 14:

> "**Figure 6:** Progression of RA-like inflammatory disease in **homozygous human C5aR knock-in and wild-type mice**. The left panel shows the increase in ankle thickness after K/BxN serum was injected i.p. on days 0 and 2. The right panel shows the clinical score. Mice #10 and #25 are homozygous for the human C5aR gene, mouse #38 is a wild-type littermate. Ankle thickening and clinical disease developed in both wild-type and homozygous hC5aR gene mice in the typical fashion described for this model (Lee et al (2002) Science, 297, 1689-1692)." [emphasis added]

The Applicants note that the phrase "in the K/BxN model" as used on page 62 at lines 20-27 is a discussion of the results obtained in Example 6 and therefore describes the results of injecting homozygous C5aR and wild-type control mice with K/BxN sera as described on page 60.

Response to (ii)

The Examiner seems to have misunderstood the results presented in Figure 7 and discussed on page 61, lines 29 to 35 which relate to testing of the claimed mouse models with a known anti-C5aR antibody designated 7F3. The Examiner asserts that it was already known that the anti-human C5aR antibody (7F3) targeted hC5aR and not mouse, so the controls required to identify compounds that specifically target hC5aR using the mice claimed are not described by the Applicants.

The specification shows that by injecting MAb 7F3 (an antibody known to bind human C5aR) into hC5aR transgenic mice injected with the K/BxN serum, inflammation was reduced. When another antibody (which does *not* bind human C5aR) was injected, there was no reduction in inflammation. In light of these results it would be clear to a person skilled in the art s/he could administer any test compound to the hC5aR transgenic mice that had been injected with K/BxN serum and determine the effectiveness of that test compound by measuring the level of inflammation in the mice compared to suitable controls.

In particular, a person skilled in the art would understand from these results that a potential novel compound that antagonizes C5aR could be tested by simply administering that compound to the hC5aR transgenic mice that had been injected with K/BxN serum (hC5aR mice of the K/BxN model) and using

as a control, **for example, an antibody of the same isotype but irrelevant specificity. This is suggested in the specification at page 62, lines 23-24.**

It is true that the anti-C5aR antibody 7F3 was already known to bind to human C5aR. However, this is precisely why it was used to verify that the mouse model described in the application is suitable for screening antagonists of C5aR. It would be clearly understood by a skilled artisan that in light of these results, the mouse model could be used to screen for new compounds that target C5aR. The Examiner's statement that the alleged use is merely using the knockin to screen anti-inflammatory compounds already known to target human C5aR is therefore incorrect.

The results presented in the specification clearly show that two test compounds (i.e., anti-C5aR antibody 7F3 and a control antibody) had different effects on the course of inflammatory disease in the model. It would be clear to those skilled in the art that the transgenic mice could be used in a similar manner for testing candidate compounds to identify therapeutic agents for the treatment of immunopathological conditions. This testing for novel compounds would involve no more than routine techniques that would be well known to those skilled in the art – in fact, it would involve the same or similar techniques as those presented in the experimental section of the specification for testing the effect of administration of antibody 7F3.

Withdrawal of this rejection is respectfully requested.

REJECTIONS UNDER §103(A)

The Examiner has maintained the rejection of claims 1-5, 8, 10, 13-20, 22, 27, 28, 30-35 and 40 as allegedly being obvious over Sato (Thrombosis and Haemostasis (1999) 82(2):865-869- henceforth "Sato"), Roebroek (Methods in Molecular Biology (2003) 209:187-200- henceforth "Roebroek"), Homanics (Methods in Alcohol Related Neuroscience Research (2002), pg 31-61- henceforth "Homanics"), Lester et al, (Curr. Opin. Drug Discov. and Dev. (2003) 6(5):663-639-henceforth "Lester"), Champtiaux (Curr. Drug Targets, CNS & Neuro. Dis.(2002)1:319-330- henceforth "Champtiaux"), Girardi et al., (J.Clin. Invest. (2003) 112(11):1644-1654- henceforth "Girardi") in view of Burmer et al., (WO 02/61087- henceforth "Burmer").

The Examiner maintains that the claims are obvious in light of the cited prior art providing knock-in techniques and the sequence of C5aR. In particular, the Examiner maintains that a person skilled in the art would have been motivated to knock-in the human C5aR gene to test functional redundancy and, that without evidence to the contrary, the results obtained would have been expected.

This rejection is respectfully traversed as applied and as it may be applied to the claims as now pending.

The Court in *KSR* repeatedly emphasized that an obviousness inquiry must take into account the predictability of the field:[1]

> If a person of ordinary skill can implement a predictable variation, §103 likely bars its patentability. For the same reason, if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill. *Sakraida* and *Anderson's-Black Rock* are illustrative—a court must ask whether the improvement is more than the predictable use of prior art elements according to their established functions.

(emphasis added)

The references upon which the Examiner relies for this rejection are merely a collection of papers describing techniques for generating knock-in or knock-out mice, along with a paper providing the human C5aR sequence.

The Applicants contend that at the time of the claimed invention, the ordinarily skilled artisan would not expect that human C5aR signaling would be activated by mouse C5a. The Applicants' assertion is supported by evidence in the form of the Declaration under 37 C.F.R. §1.132 by Dr. Craig Gerard (the "Gerard Declaration").

Dr. Gerard has over 28 years of experience in research involving C5a and its receptor. Thus, Dr. Gerard qualifies as a person of ordinary skill in the art of C5a and its receptor. In the Gerard Declaration, Dr. Gerard explains why, at the time of the invention, it was not predictable that murine C5a would activate the human C5aR expressed *in vivo* in a knock-in mouse. Below, the Applicants present a summary of the reasoning provided by Dr. Gerard in the declaration as to why a person of ordinary skill in the art would find the results presented by the Applicants unpredictable and unexpected:

i. Mouse and human C5a and the receptors are divergent in amino acid sequence. See Exhibit-2 which shows that the mouse and human C5a are

[1] *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 13 (U.S. 2007)

only 63.5% identical and Exhibit-3 which shows that the mouse and human C5aR are only about 30% identical.

ii. Cross species functions of anaphylatoxin ligands, such as C5a, and their respective G protein coupled receptors, were well known to be unpredictable because although human C4a was shown to activate anaphylactic signaling by interacting with guinea pig C3aR, human C4a did not interact at all with human C3aR. (Lienenklaus *et al.* (1998) *J Immunol*, 161:2089-2093, Exhibit 6).

iii. Based on the above, Dr. Gerard concludes that one of skill in the art would have believed that mouseC5a would be unlikely to bind to and effect signaling of human C5aR.

In the Gerard Declaration, Dr. Gerard also discusses that the references cited by the Examiner would not teach or reasonably suggest to a person of skill in the art that murine C5a would activate the human C5aR expressed *in vivo* in a knock-in mouse. A summary of this discussion in the Gerard Declaration is provided below:

i. Cain presents the observation that a known human C5aR antagonist (F-[OPchaWR]) has approximately 1000-fold less affinity for mouseC5aR (see abstract, first sentence). The authors hypothesize that a possible cause of this difference is the species-species *variation* in the sequence of the activation domains of C5aR (see first paragraph of the Discussion, page 1575). Cain therefore provides a clear example of a ligand that has high affinity for human C5aR but which exhibits wide variations in binding affinity to C5aRs from other species further supporting the conclusion that a ligand that binds to mouse C5aR (e.g. Mouse C5a) would not bind to and effect signaling of human C5aR.

ii. The Drago paper does not provide any evidence of a humanized receptor binding to a mouse ligand. The paper looks almost exclusively at the phenotype of knock-out mice in which specific subunits of neuronal nicotinic receptors have been deleted.

iii. The Gu paper relates to a study of the Neuropilin-1 (Npn-1) receptor which binds multiple ligands from structurally distinct families such as semaphorins (Sema) and vascular endothelial growth factors (VEGF). There is no mention at all of comparison of ligand binding across different species, and no evidence of a humanized Npn-1 receptor binding to a mouse ligand.

iv. The Gu paper relates to a study of the Neuropilin-1 (Npn-1) receptor which binds multiple ligands from structurally distinct families such as semaphorins (Sema) and vascular endothelial growth factors (VEGF). There is no mention at all of comparison of ligand binding across different species, and no evidence of a humanized Npn-1 receptor binding to a mouse ligand.

Dr. Gerard concludes that there is nothing in the cited prior art to suggest that a skilled person could have predicted that mouse C5a would bind to human C5aR and that on the contrary, a skilled person would not have predicted success in making a mouse that is transgenic for human C5aR and in which C5a endogenous to the mouse binds to and effects signaling of the human or humanized C5aR.

In the Gerard Declaration, Dr. Gerard states that the results provided in the application were surprising and unexpected. Specifically, Dr. Gerard states that he would not have expected murine C5a generated *in vivo* in the K/BxN model of arthritis would function at the human C5a receptor.

In view of the lack of predictability of the human C5aR signaling being activated by mouse C5a, one of skill in the art would have no reason to generate transgenic mouse with humanized C5aR.

For at least these reasons, the Applicants submit that the rejection of the claims under §103(a) should be withdrawn, which action is respectfully requested.

CONCLUSION

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number RICE-050.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: July 1, 2010

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Enclosure(s): Information Disclosure Statement
SB/08A form
Takeuchi *et al.*, Biochem. and Biophys. Res. Comm. 293: 953-957 (2002)
Dymecki (Proc Natl Acad Sci U S A. 93(12): 6191–6196 (1996)
Declaration under 37 C.F.R. §1.132 by Dr. Craig Gerard

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